Effects of Fungicides on Microbial Populations In Soil

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Summary

The ecological significance of any temporary change in the composition of soil populations can only be judged when the role of groups of soil organisms within the terrestrial ecosystem is properly defined and quantified. A proposal is made to approach the problem with special emphasis on the selective inhibition of bacterial and fungal activity.

Possible interruptions of the energy flow, or blockage of essential functions within the ecosystem, are important criteria for the secondary effects of fungicides. A well oriented research program in biochemical ecology in combination with the methods of systems analysis should lead to the identification of the system components with the highest sensitivity. Examples are given for an attempted approach with the fungicide captan and the utilization of glucose, cellulose and cutin.

Most soil fungi are enzymatically well equipped. The elimination or reduction of fungicide-sensitive members generally triggers a sequence of replacements within the population, resulting in the preservation of the metabolic integrity. In this context the principles underlying a combined approach of chemical and biological control of parasitic soil fungi are discussed. It is concluded that soil fungicides *sensu stricto* act as regulators of a disturbed biological balance.

Introduction

Variable amounts of fungicides are applied to the soil either by direct incorporation (soil fumigants, soil fungicides, seed dressings) or as run-off deposits from spray applications. In any case most of these chemicals are designed to act against pathogenic fungi. It must be expected that they affect not only the pathogens but, also, soil microbes as a whole. In this respect, soil treatment with fungicides differs considerably from the direct control of fungal "mono-cultures" on the above-ground parts of crop plants. Moreover, any side-effects of soil fungicides occurring below ground must occur at concentration ranges at or below the usual application rates. In this respect they differ considerably from insecticides or herbicides (Domsch, 1963).

In view of the complexity of terrestrial ecosystems, it would be a more appropriate approach to discuss the side-effects of fungicides on the total soil ecology rather than certain isolated aspects. However, the actual accomplishments in this direction are minimal. Most of the investigations have concentrated on numerical shifts in populations or changes in certain physiological activities, as nitrification, ammonification or cellulose decomposition, to name some of the frequently occurring criteria. Only recently have biologists become more and more "ecology-conscious". Moreover, modern soil biology is in the unsatisfying position of using descriptive methodology for the interpretation of functional relationships. So far, only preliminary attempts have been made to quantify specific biochemical activities of soil organisms in such a way that the components of the soil ecosystem can be weighed against each other.

According to the present state of knowledge, this paper will concentrate on microbial populations. We are aware that the exclusion of nematodes and protozoa from the discussion is a serious shortcoming since manifold interrelationships exist between these groups on one side and microorganisms on the other. How and to what extent the regulating functions of nematodes and protozoa are affected by fungicides remain to be investigated.

The following pages summarize some results from previous and more recent studies with special emphasis on the selective inhibition of fungi and bacteria, the critical interruptions of functions in the carbon cycle, the restitution of metabolic integrity of microbial populations and the integrated approach for fungicide application.

Selective Inhibition

Techniques for the enumeration of soil microorganisms are based on the "development" of viable units in or on proper nutrient media. The only statement that can be made from this type of analysis is that of the presence or absence of organisms. In recent years a number of new methods have become available for a more meaningful characterization of vegetative stages of soil fungi (calculation of hyphal length per g soil, plating of "washed" soil particles, immersion

tubes and others). However, none of the techniques gives any information about the activity of the propagule, nor of the relative participation of groups of microbes in specific biochemical activities.

One should be able to distinguish first of all between the share that bacteria and fungi have in the metabolic activity of a particular soil. In addition, the determination of population shifts after fungicide application should be done without the disintegration of the whole system. One approach is the selective inhibition of bacteria and fungi with antibiotics. A total of thirteen antibiotics (tetracyclin, chlorotetracyclin, chloramphenicol, erythromycin, neomycin, polymyxin, streptomycin, novobiocin, endomycin, pimaricin, griseofulvin, nystatin, cyloheximide) have been tested at concentrations between 500 and 2000 ppm (Domsch, 1968).

Most of them had a life-time too short (<50 hrs) for further experimentation. Four antibiotics (cycloheximide, nystatin, streptomycin and novobiocin) passed the stability test and were further tested for specificity at the relatively high concentration that must be used in soil applications. An example of the specificities shown by cycloheximide and streptomycin is given in Table 1. Nystatin and novobiocin failed these tests. Though they have a full inhibitory action against the one group they were designed for, they detoxified the inhibitor.

Table 1. Selective inhibition of fungi and bacteria in vitro.

	Cyclo-heximide 1000 p.p.m.	Strepto-mycin 2000 p.p.m.
Mortierella alpina	0	102
Trichoderma viride	0	120
Rhizoctonia solani	0	100
Aspergillus fumigatus	0	96
Phoma eupyrena	0	89
Cladosporium cladosporioides	0	100
Phialophora fastigiata	0	94
Mucor hiemalis	0	81
Fusarium solani	0	104
Bacillus cereus var. mycoides	100	0
Bacillus subtilis	100	0
Mycobacterium smegmatis	100	0
Bacillus sphaericus	100	0
Pseudomonas cruciviae	100	0
Chromobacterium violaceum	100	0
Corynebecterium michiganense	100	0
Agrobacterium radiobacter	100	40
Pseudomonas fluorescens	100	0
Sarcina flava	100	0

Finally, cycloheximide and streptomycin were used in experiments with sterile soil which had been inoculated with pure cultures of soil fungi (*Phialophora fastigiata, Fusarium solani, Cylindrocarpon destructans, Mucor hiemalis, Aspergillus fumigatus, Phoma eupyrena, Mortierella alpina, Rhizoctonia solani*) or bacteria (*Pseudomonas fluorescens, Bacillus subtilis, Bacillus cereus* var. *mycoides, Chromobacterium violaceum, Sarcina flava, Corynebacterium michiganense, Nocardia citrea*). The 0_2-uptake was manometrically determined for each single species within a 120 hr-period in the presence and absence of 1000 ppm cycloheximide and 2000 ppm streptomycin, respectively. It showed that 0_2-uptake can be used within limits as a suitable measure of inhibitory effects. Further experiments demonstrated that 0_2-uptake of a remoistened air-dry soil is predominantly due to the metabolic activity of soil bacteria (Fig. 1).

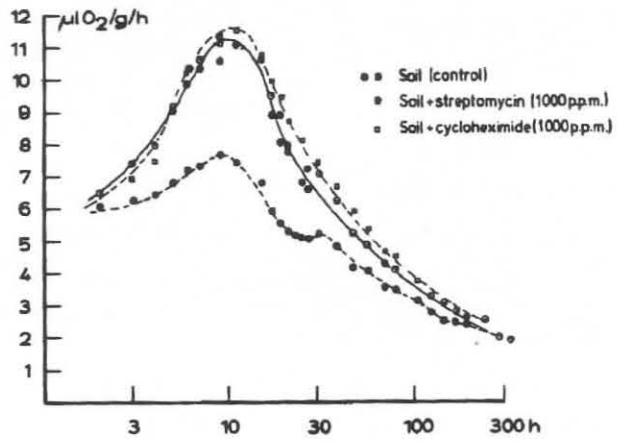

Fig. 1 Influence of streptomycin and cycloheximide on the O_2 uptake by samples of re-moistened air dry soil.

In order to broaden the basis for further experimentation we tested more chemicals with a selective fungicidal action. More than 50 experimental fungicides were provided by the Bayer Biological Laboratories. They had high fungitoxicity at low concentrations *in vitro* and good performance as soil fungicides in greenhouse tests. In concentrations of 10, 20, 50, 100 and 200 ppm they were added to remoistened soil and the 0_2-uptake measured in short intervals for a length of 70 hours. Basically, 4 types of response were obtained (Fig. 2): (1) Complete inhibition of 0_2-uptake, (2) short-term inhibition followed by recovery with no significant difference in the total 0_2-uptake in fungicide-treated and untreated soils, (3) initial inhibition followed by a manifold increase in 0_2-uptake indicating fungicide decomposition, (4) no influence on 0_2-uptake indicating the lack of toxic side-effects on bacteria. Five chemicals were selected for further tests (Table 2). The next screening will determine whether they can be used for the selective inhibition of fungi in general, or even of special groups of fungi (e.g., basidiomycetes, phycomycetes).

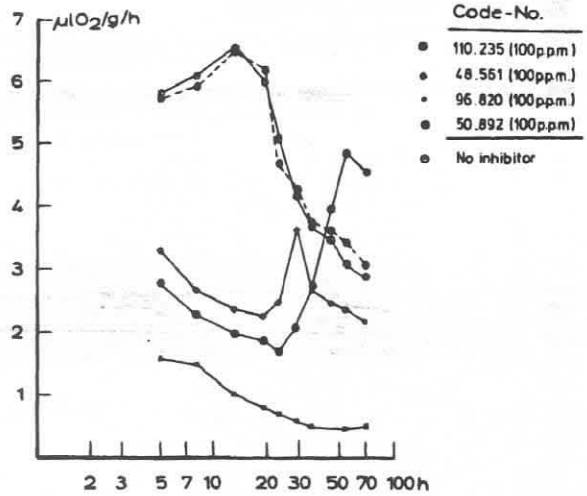

Fig. 2 Influence of 4 types of experimental fungicides on the O_2 uptake by soil.

Table 2. Fungitoxicity of experimental inhibitors.

Code-No.	In vitro	In Soil	Remarks
66.525		2.5 p.p.m.	selective (Rhiz.)
106.603		50 p.p.m.	selective (Rhiz.)
91.395		100 p.p.m.	selective (Rhiz.)
102.512		100 p.p.m.	selective (Pyth.)
110.235	< 10 p.p.m. (broad)	100 p.p.m.	(Fus.)

Critical Interruptions

The primary production by plant communities in connection with subsequent energy flow through the ecosystem should be of primary concern in discussing fungicides and soil ecology. The main questions in our context are as follows: (1) Which components of the decomposer system are most essential for maintaining the energy flow? (2) Are fungi the dominating group of decomposers as in forest soils, or bacteria as in most arable soils? (3) Can the succession of decomposing microorganisms be interrupted, leaving plant residues in a "reduced" state? (4) Which are the most sensitive catabolic activities performed by microorganisms in the soil? (5) How long may inhibitory effects persist?

Although we are not now in a position to answer these question satisfactorily, a first approximation with simple models can be attempted.

The incorporation of a well defined energy substrate into the soil can be regarded as a suitable starting point. The survey of some typical plant and animal residues (Fig. 3) shows a succession of decomposition maxima. The application rate necessary for the elaboration of a significant net 0_2-uptake was between 200 ppm (glucose, starch) and 5000 ppm (cellulose, chitin). Experiments with a preparation of native lignin failed to give any response in 0_2-uptake during a 1000 hour incubation period. This showed the extremely low decomposition rate of this material.

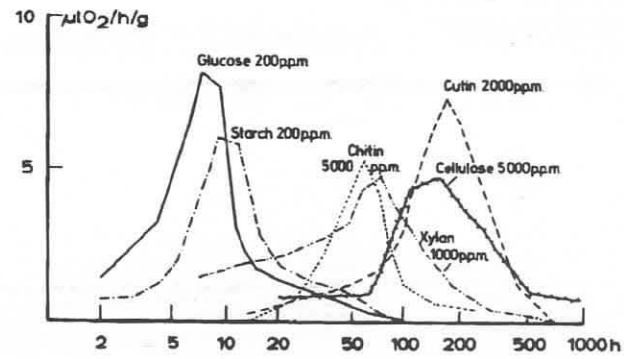

Fig. 3 Net O_2 uptake by soil samples supplemented with 6 different carbohydrates.

In a series of experiments, the influence of captan at different application rates on substrate utilization by microbes was studied. Captan was chosen because of its high stability. 0_2-uptake of the treated soil samples again was taken as an overall indicator. Some typical results from a total of 8 different substrates (glucose, starch, pectin, aesculin, xylan, cellulose, cutin, chitin) are given in Fig. 4, 5, and 6. The addition of 200 ppm glucose (Fig. 4) leads to a respiration peak 8-10 hours after application. Captan caused a short period (15 hr.) of depressed respiration.

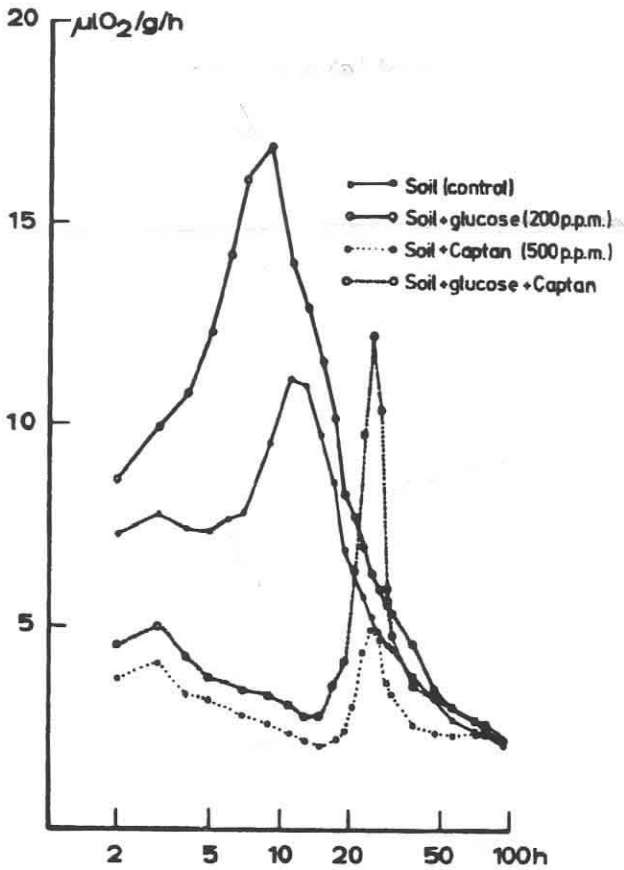

Fig. 4 Action of captan on soil respiration in the presence or absence of glucose.

The decomposition of cellulose (Fig. 5) requires the production of cellulases and their action in the ambient medium before 0_2-uptake can be expected to increase. The calculations indicate that after 50 hours the net respiration rates steadily increase and reach a maximum after 150 hrs in the absence of captan. In the presence of 200 ppm captan the maximum is shifted to 500 hrs. With 500 ppm captan there was a complete inhibition of cellulose decomposition for at least 6 weeks after application.

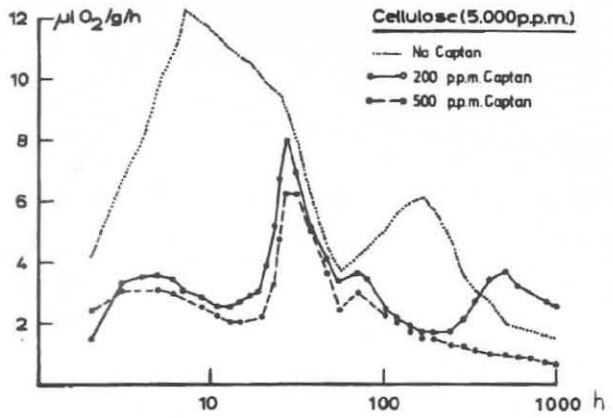

Fig. 5 Action of captan on soil respiration in the presence of cellulose.

An even more severe influence can be observed in the case of cutin utilization (Fig. 6). So far, only fungi are known to decompose cutin. This might be the reason for the high sensitivity of this process. From all that is known about the mode of action of this fungicide it seems unlikely that captan has a specific influence on the cutinase-system.

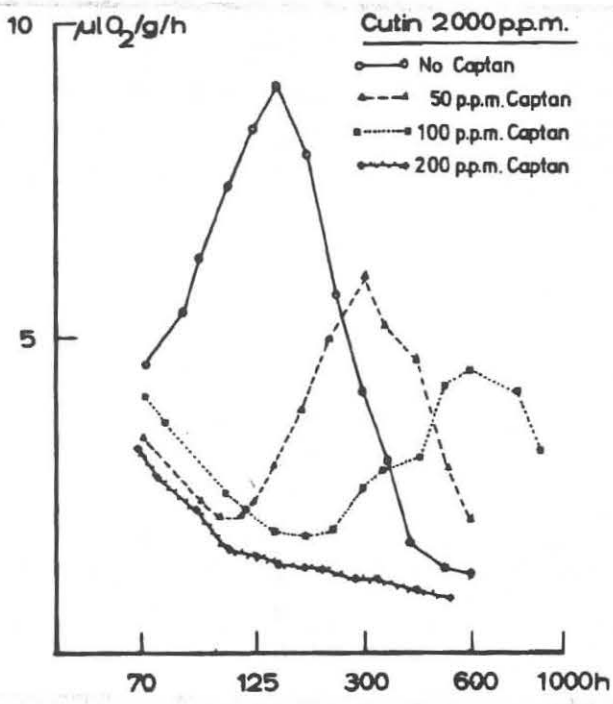

Fig. 6 Action of captan on soil respiration in the presence of cutin.

The time required to overcome inhibitory effects varies from one day to several months. If the theoretical amount of non-oxidized carbon sources in fungicide-treated soil is calculated at the stage of a restituted equilibrium between treated and non-treated soil the following results will be found (Table 3): (1) Components of the ecosystem which attack more resistant substrates are of particularly high sensitivity. (2) Zymogenic organisms are either highly resistant, adaptable or replaceable.

Table 3. Percentage of non-oxidized carbon sources remaining in a captan-treated soil.

Captan concentration (p.p.m.)	Cutin 2000 p.p.m.	Cellulose 5000 p.p.m.	Pectin 500 p.p.m.	Glucose 200 p.p.m.
50	10	–	–	–
100	55	–	–	–
200	100	11	–	–
500	–	100	59	28

Metabolic Integrity

The reactivity of a soil micropopulation in the presence of fungicidal substances ranges from high susceptibility to high tolerance. Unless reactivity is specifically coupled with catabolic activities, it must be assumed that the elimination of species with distinct potencies for substrate utilization is compensated by the survival of other, more resistant and equally potent species. A compilation of results on this point is given in Table 4. The 8 fungi were selected from 20 species, the percentage of which within the total population of recorded fungi was significantly reduced by each of 6 different fungicides (Domsch, 1960a). The information about the intensity of substrate decomposition was taken from a systematic screening of metabolic potencies (Domsch, 1960; Domsch et al., 1969).

Some of the fungi which are drastically reduced by

Table 4. Potential of soil fungi with high fungicide sensitivity to decompose carboxymethylcellulose, xylan, pectin, and chitin in vitro.

Fungus	CMC	Xylan	Pectin	Chitin
Acremonium furcatum	(+)	++	(+)	0
Fusarium avenaceum	+	+	(+)	0
Fusarium dimerum	+	++	(+)	0
Verticillium nigrescens	+	+	(+)	0
Mortierella alpina	0	0	(+)	++
Mortierella exigua	0	0	0	+
Mortierella stylospora	0	0	0	+
Mucor hiemalis	0	0	+	+

0 = no decomposition
(+) = slight decomposition
\+ = moderate decomposition
++ = high decomposition

soil fungicides can be characterized by enzymatic potencies of wide (carboxymethycellulases, pectinases) and limited (chitinase) distribution. But for each of these species, other frequently occuring soil fungi may take over a "lost" function. This applies in particular to the high fungicide tolerance of *Chaetomium* and *Penicillium* species and their rich complement of degrading enzymes (Domsch, 1960a). Chitinolytic fungi, which belong to the phycomycetes, are highly fungicide sensitive. They are probably not immediately replaced by other fungi. But it is very likely that the integrity of "soil metabolism" is preserved in this case by streptomycetes and/or bacteria.

We are well aware that this type of approach leads to nothing better than an approximation of what happens in soil in reality. Nevertheless, the following statements might be allowed: soils vary in their microbial composition; we have no reason to give preference to any specific species composition (with the exception of undesired plant pathogens); we know that species are eliminated or reduced by fungicides as well as by extreme drought or flooding – to call the attention to just two of many environmental factors. We therefore should agree that the maintainance of functions is more important than a "reference" species composition. All the results so far obtained point to a surprisingly fast restitution of affected functions. This can be regarded as part of the biological "buffering capacity" of soils.

Integrated Approach

The term "integrated approach" is adopted from entomology, meaning the combination of chemical and biological control of insects. A number of different mechanisms (antibiosis, competition, lysis, microparasitism) are known to operate in the biological control of soil-borne pathogens. A key factor in the characterization of an infested soil or in the suppression of plant diseases is the inoculum density. Quantitative relationships between inoculum density and the dosage of a fungicide required for disease control, have been demonstrated that fit models derived from theoretical considerations (Baker, 1968).

We know that infective propagules of many pathogenic fungi are widely distributed in soils, but they do not cause diseases as long as the inoculum density stays below a critical level. There is good reason for the assumption that soil fungicides *sensu stricto* (Domsch, 1964) are acting as regulators in shifting the equilibrium between saprophytes and parasites with the final result of a non-critical inoculum density of pathogens.

This hypothesis offers an explanation for the failure of soil fungicides (not fumigants) to kill most pathogens even at extremely high application rates to soil. On the other hand the regulating function requires a saprophytic fungus flora less sensitive to fungicides than parasitic components. Experimental data gained in a comparative study of each 24 saprophytic and parasitic soil fungi indicated the validity of this assumption. With captan the ED^{50} varied from 14-692 ppm with an average at 104 ppm for saprophytes. The corresponding data for parasites was 9-104 ppm with an average at 37 ppm (Domsch, 1959).

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